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Keratin Subtypes in Carcinomas of the Uterine Cervix: Implications for Histogenesis and Differential Diagnosis

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ABSTRACT

Normal epithelia and carcinomas of the human uterine cervix were studied by monoclonal antibodies chain specific for cytokeratins 4, 8, 10, 13, 14, 18, and 19. Most cells in 13 examined squamous carcinomas revealed a cytokeratin phenotype detected in ectocervical basal cells and endocervical subcolumnar reserve cells: 8^+, 14^+, 18^+, 19^+, 4^-, 10^-, 13^-. We propose that these two cell types are closely related or identical and that squamous carcinoma of the cervix originates in this cell type. In more differentiated tumor cells cytokeratins 4, 10, and 13, which are present in suprabasal layers of the normal ectocervical epithelium, were coexpressed with basal cell cytokeratins. Thus, contrary to previous beliefs, all cytokeratins detected in carcinomas were also present in normal epithelium of uterine cervix.

The cytokeratin profile of cervical adenocarcinomas corresponded to that of columnar endocervical cells (8^+, 18^+, 19^+), although two of the three adenocarcinomas also expressed cytokeratin 4, which in the normal endocervix was detected in scattered single columnar cells only. The new monoclonal antibody DE-K14, specific for cytokeratin 14, proved a specific marker of subcolumnar reserve cells in the endocervix. It was also the only one that reacted with all cervical squamous carcinomas but with none of the cervical adenocarcinomas and, as such, has a potential value for pathological differential diagnosis of cervical tumors.

INTRODUCTION

It is often difficult to determine from which cells a tumor is initially derived. Recent studies have shown that typing and subtyping of intermediate filaments can facilitate to define the origin of malignant cells (1–4).

The major class of epithelial intermediate filaments, cytokeratins, comprises a group of at least 19 different multigene derived proteins, which are expressed in different epithelia in specific combinations (5–9) as heteropolymers (10–13). The cytokeratin pattern of one-layered "simple" epithelia and glandular epithelia is relatively simple, comprising cytokeratins 7, 8, 18, and 19 (5, 8, 14). Most stratified epithelia display a much greater complexity of cytokeratin polypeptides (5, 8, 9, 15–18), and within these stratified epithelia, the various cytokeratin proteins are differentially expressed in basal, intermediate, and superficial layers (16, 17, 19–22). Cytokeratin characteristics of normal epithelia are usually also expressed in the tumors that originate from these epithelia (8, 23–25). Consequently, mAbs[1] recognizing individual cytokeratin polypeptides provide a valuable tool in the study of tumor histogenesis.

We have used a panel of monoclonal antibodies that are chain specific for individual cytokeratin proteins in order to compare expression of these cytokeratins in normal epithelia of the uterine cervix with patterns of expression in cervical carcinoma. We report that the basal cells of ectocervix, endocervical subcolumnar reserve cells, and most cells of squamous carcinomas have a similar cytokeratin phenotype, which differs from cer-

vical adenocarcinomas; the latter reveal the cytokeratin phenotype of endocervical columnar cells.

MATERIALS AND METHODS

Antibodies. Chain-specific monoclonal antibodies for CK proteins are listed in Table 1. mAb DE-K14 was obtained after a fusion of SP2/0 myeloma with splenic lymphocytes from a BALB/c × C57BL/6 F_1 (hereafter CB6F$_1$) mouse which had been immunized with a cytoskeletal preparation extracted from human ectocervical epithelium by detergent/high salt extraction, as described previously (23). Hybridoma supernatants were screened on frozen sections of human uterine cervix. mAb DE-K14 originated from a well showing reactivity with basal cell layer of ectocervical epithelium. Colonies from this well were then cloned twice by limiting dilution. Ascites fluid was prepared in pristane primed CB6F$_1$ mice. Immunoglobulin type of DE-K14 antibody was determined by the Ouchterlony immunodiffusion technique as IgM.

Antiserum monospecific for CK14 was obtained from a CB6F$_1$ mouse immunized with a synthetic tetradecapeptide corresponding to the carboxy-terminal sequence KVVSTHEQVLRTKN of CK14 [identified by Marchuk *et al.* (26)] conjugated to keyhole limpet hemocyanin (Cambridge Biochemicals, Cambridge, England). The specificity of the antiserum to the synthetic peptide (uncoupled to keyhole limpet hemocyanin) was tested by "dot blotting." As a control, synthetic peptides corresponding to carboxy-terminal sequences of several other human cytokeratins (CK7, CK16, CK17) were used (prepared on the basis of data from Refs. 27, 28, and 29, respectively). The antiserum reacted with a synthetic peptide specific for CK14 and not with synthetic peptides specific for other cytokeratins. It will be referred to further as anti-CK14 serum.

Other CK specific mAbs used were: Ks 8.12, specific for CK13 and CK16 (30); RCK 102, specific for CK5 and CK8 (31); and CAM 5.2, originally described as specific for CK (*M*, 50,000, 43,000, and 39,000) (32) but later found to be specific for CK8 only.[2] Antibodies Ks 8.12 and Km 4.62 were purchased from BioMakor (Westburch Biochemie), and CAM 5.2 was from Becton Dickinson; other monoclonal antibodies were either produced in our laboratory (designated DE-) or kindly donated by their authors (see Table 1).

Immunohistochemistry. An indirect immunofluorescence technique, using fluorescein-conjugated goat anti-mouse immunoglobulin as a second antibody, was used on cryostat sections of human tissues. Nonspecific binding of secondary antibody was prevented by preincubation of slides with normal goat serum (10% in PBS). First antibodies were applied for 1 h at room temperature, second antibody (goat antimouse fluorescein isothiocyanate; Nordic Diagnostic, Tilburg, The Netherlands) was applied for 30 min. Slides were mounted in watersoluble mounting medium containing polyvinyl alcohol (Janssen), glycerol, and an anti-bleach agent, *n*-propyl gallate (Sigma). In parallel, an indirect immunoperoxidase method was used as described previously (33).

Gel Electrophoresis and Immunoblot Technique. Cytokeratin-enriched cytoskeletal proteins were prepared from thick cryostat sections of various tissues by a procedure described by Chin *et al.* (34). One-dimensional gel electrophoresis was performed in 7.5% polyacrylamide slab gels, as described by Laemmli (35). Two-dimensional electrophoresis was performed essentially as described by O'Farrell *et al.* (36). In the first dimension, isoelectric focusing was performed in 5% polyacrylamide rod gels containing 2% ampholines, pH 3.5–10 (0.8%, pH 4–6; 0.8%, pH 5–7; 0.4%, pH 3.5–10) (Bio-Rad, Richmond, CA). For the

Table 1 *Chain-specific mAbs used in this study*

Antibody	Specificity	Refs.
M6B10	CK4	17
M20	CK8	54
DE-K10	CK10	33
IC7	CK13	17
DE-K14	CK14	This paper
RCK 106	CK18	64
Km 4.62	CK19	20

second dimension, rod gels were applied directly onto the stacking gel of sodium dodecyl sulfate-polyacrylamide gels. Electrophoretically separated polypeptides were transferred to nitrocellulose paper (membrane filters BA 85; Schleicher and Schüll, Dassel, Federal Republic of Germany) by semidry blotting for 1 h at 250 mA (2117 Multiphor II electrophoresis unit; LKB-Producter AB, Sweden). Buffers used were: 40 mM norleucine/25 mM Tris, pH 9.4, for cathodic paper and gel; 25 mM Tris/20% methanol, pH 10.4, for nitrocellulose sheet; and 300 mM Tris/20% methanol, pH 10.4, for anodic paper. After preincubation with 3% low fat milk in PBS, blots were incubated with culture supernatants, diluted when necessary in PBS containing 0.05% Tween 20 and 1% normal goat serum. The extent of reaction was visualised either by Auro Probe BL plus goat anti-mouse immunoglobulin (Janssen) or ^{125}I-labeled goat anti-mouse immunoglobulin.

Tissues. Tissue from normal uterine cervix was obtained from hysterectomies performed because of benign disorders not affecting the uterine cervix. Fourteen specimens of invasive SQC and three invasive adenocarcinomas of the uterine cervix were studied. The age of the patients varied from 23 to 80 years. Pathological classification, Federation of International Gynecologists and Obstetricians stage, and the differentiation grade of tumors are given in Table 3.

The samples were snap-frozen in liquid nitrogen and stored at −70°C until use.

Cell Lines. A-431 [epidermoid carcinoma of the vulva; CK proteins detected: 5, 6, 7, 8, 13, 14, 15, 16, 17, 18 (5)]. TR146 (squamous cell carcinoma of buccal mucosa that had infiltrated a lymph node (37); CK proteins detected: 5, 6, 8, 14, 17, 18).³ TR146 line was obtained from the laboratory of Professor Franke, Institute of Cell and Tumor Biology, Heidelberg, Federal Republic of Germany.

RESULTS

Characterization of mAb DE-K14

Reactivity Pattern on Normal Human Tissues. By immunofluorescence microscopy, fine, intracytoplasmic fibrillary staining

³ W. W. Franke, personal communication.

compatible with keratins was obtained with antibody DE-K14 in all cells of TR146 cultures (Fig. 1a) and in many but not all cells of A-431 cultures. When examined on cryostat sections of various human tissues it stained basal cells of nonkeratinizing stratified epithelia (ectocervix, esophagus, tongue, vagina), basal and spinous layers of the epidermis, myoepithelial cells, sweat gland ducts, and basal cells in the crypts of the ileum.

Specificity Determined by Immunoblotting Analysis. Western blot analysis using various preparations of cytoskeletal polypeptides from different tissues and cell lines revealed specific reactivity of this antibody with CK14 (M_r 50,000). This is illustrated on immunoblot of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cytoskeletal proteins isolated from squamous cell carcinoma of the uterine cervix (Fig. 1b) and immunoblots of two-dimensional gels of cytoskeletal proteins isolated from the TR146 cell line (Fig. 2, e and f), squamous cell carcinoma of the vulva (Fig. 2, a and b), and squamous cell carcinoma of the uterine cervix (Fig. 2, c and d). On some immunoblots DE-K14 weakly stained additional spots which we regard as degradation products of CK14 (not shown).

Cytokeratin Expression in Normal Cervical Epithelium. Two morphologically distinct types of epithelium are present in the normal human uterine cervix: nonkeratinizing squamous epithelium lining the ectocervix; and a single layer of mucus-secreting columnar epithelium lining the cervical canal and its glands. Close to the so-called squamocolumnar junction, where these two epithelia meet, a single layer of cuboidal cells is seen between columnar endocervical cells and basal lamina, the SRC.

The cytokeratin expression of individual cytokeratins detected in these different epithelia by chain-specific mAbs is summarized in Table 2. In the endocervix, both columnar cells and SRC contained simple epithelial-type cytokeratins 8 and 18, as detected by mAb M20 and mAb RCK 106, respectively (Fig. 3, a–c) and by antibody specific for CK19 (mAb Km 4.62). Neither columnar cells nor SRC expressed CK10 (mAb DE-K10) or CK13 (mAb 1C7). Single columnar cells in different positions within the endocervix were stained with mAb M6B10, specific for CK4 (Fig. 3e); the other columnar cells and all SRC were negative. Only one antibody could distinguish between SRC and columnar cells, namely mAb DE-K14, specific for

Fig. 1. (a) Immunostaining of cytokeratin filament in TR146 cells with mAb DE-K14. (b) Immunoblot of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cytoskeletal proteins from the squamous cell carcinoma of the cervix uteri.
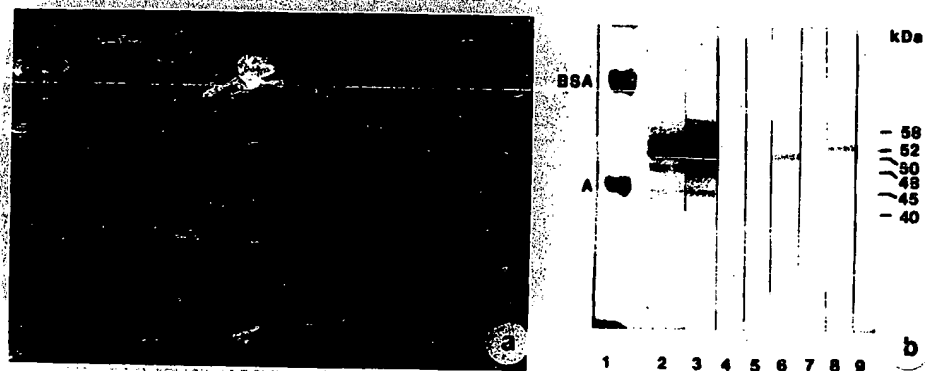
Lanes		CK	M_r
1. Molecular weight markers	Staining		
2. Cytoskeletal proteins	Ponceau S		
3. mAb Ks 8.12 (30)		13, 16	52,000, 48,000
4. mAb CK13 (17)		13	52,000
5. mAb DE-K14		14	50,000
6. Anti-CK14 mouse serum (see "Materials and Methods")		14	50,000
7. mAb Km 4.62 (20)		19	40,000
8. mAb RCK 102 (31)		5, 8	58,000, 52,000
9. mAb RCK 106 (64)		18	45,000

Fig. 2. Immunoblots of two-dimensional gel electrophoresis (first dimension, isoelectric focusing; second dimension, SDS-PAGE) of cytoskeletal proteins isolated from squamous cell carcinoma of the vulva (a, b), squamous cell carcinoma of the cervix uteri (c, d), and TR146 cell line (e, f). Ponceau S (a, c, e), mAb DE-K14 (b, d, f).
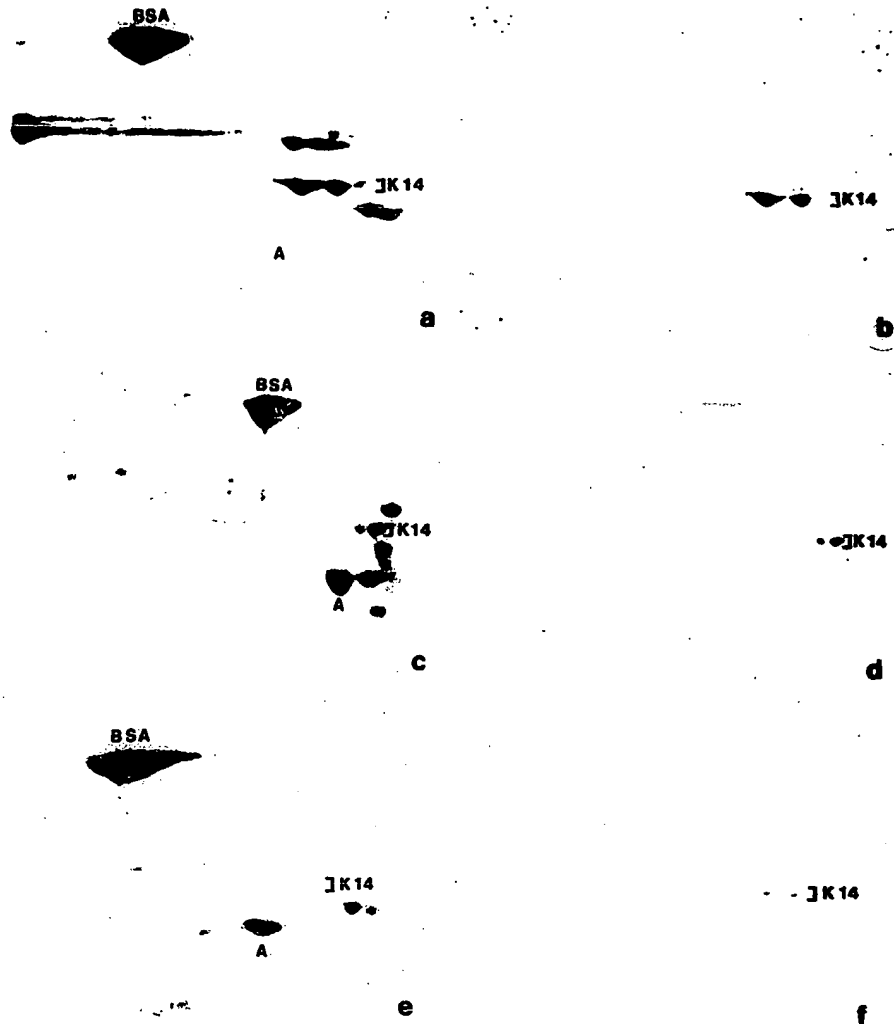
Table 2 *Cytokeratin phenotype of normal human cervix epithelium*

	CK4	CK8	CK10	CK13	CK14	CK18	CK19
Endocervix							
Columnar cells	−/+[a]	+	−	−	−	+	+
Subcolumnar reserve cells	−	w[b]	−	−	+	+	+
Ectocervix							
Basal layers	−	w	−	−	+	⊢	+
Parabasal 2–3 layers	−	−	+f	+	+	−	−
Intermediate/superficial layers	+	−	+f	+	−	−	−

[a] Single cells in various localities.
[b] w, weak staining; f. variable number of positive cells.

CK14 which showed prominent staining of SRC while columnar cells were negative (Fig. 3d).

Expression of keratins in the stratified squamous epithelium of the ectocervix varied in relation to the state of epithelial maturation (Fig. 4). Basal keratinocytes were positive for CKs 8, 14, 18, and 19 while suprabasal cells expressed CKs 4, 10, and 13. Two to three cell layers immediately above the basal layer were positive for CK14 and were negative for CK4. Staining of suprabasal layers with antibody DE-K10, specific for CK10, revealed a more heterogeneous pattern. Antibody 1C7, specific for CK13, stained uniformly all layers of the ectocervix except the basal cells.

Cytokeratin Expression in Cervical Carcinomas (Table 3). The three adenocarcinomas examined revealed the cytokeratin phe-

notype corresponding to that detected in columnar endocervical cells, i.e., positive for CKs 8, 18 (Fig. 5a), and 19 (Fig. 5b) and negative for CKs 10, 13, and 14. Interestingly, most tumor cells in two of the three adenocarcinomas expressed CK4 which in the normal endocervix has been detected only in a small subpopulation of columnar cells (Fig. 3e).

All SQC expressed the cytokeratins found in basal cells of the ectocervix and in SRC, i.e., CKs 14, 18, 19, and almost always CK8. mAbs specific for CKs 14, 18, and 19 consistently showed prominent staining through all or almost all tumor cells (Table 3; Figs. 6, a and c, and 7, a, c, and e), while mAb specific for CK8 gave usually less prominent positivity (Fig. 6d). In three SQC expression of these "basal cell-type" cytokeratins was sometimes more pronounced on the periphery of tumor islands (Fig. 6, e and f). Cytokeratins emerging in the more mature, upper layers of the normal ectocervix, CKs 4, 10, and 13, were found in some of SQC tumors only. Usually a small percentage of cells was stained in more keratinized areas (Fig. 7, b, d, and f–h); these cells were sometimes negative for basal cell-type cytokeratins (see Fig. 6, e and f), but more often, expression of basal cell-type cytokeratins was not down-regulated in these areas (Figs. 6, a, c, and d, and 7, a, c, and e). In several SQC coexpression of basal cell-type cytokeratins and "maturation-related" cytokeratins (CKs 4, 10, 13) occurred in a large percentage of tumor cells (Table 3, cases 12–16).
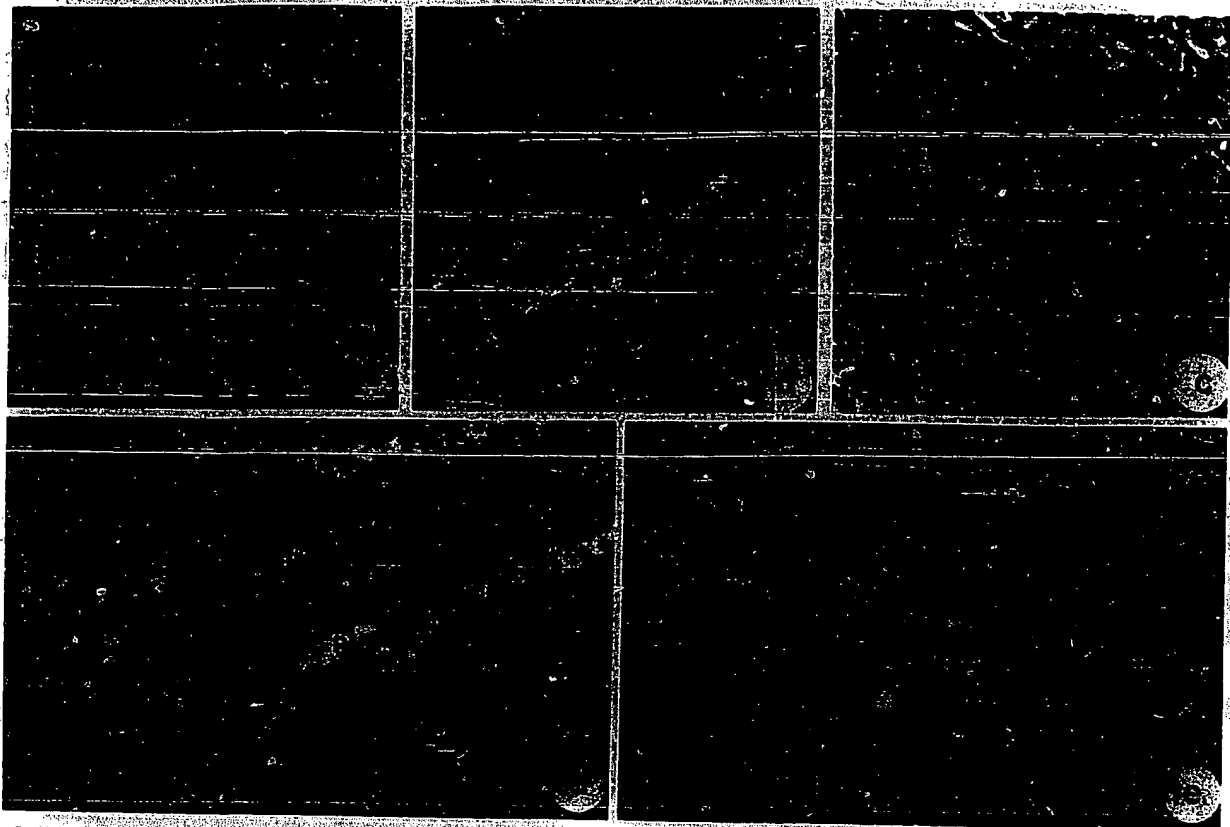
Fig. 3. Endocervical epithelium: columnar cells (C), subcolumnar reserve cells (SR), mAb RCK 106, anti-CK18 (a). mAb M20, anti-CK8 (b). Corresponding phase-contrast micrograph (c). mAb DE-K14, anti-CK14 (d). Both SRC and columnar cells are stained with mAbs RCK 106 and M20, while mAb DE-K14 stained SRC only. Single columnar cells stained with mAb M6B10, specific for CK4 (e).

DISCUSSION

We have extended previous studies on expression of cytokeratins in human uterine cervix and cervical neoplasias by application of a wider panel of monoclonal antibodies chain specific for cytokeratin polypeptides (CKs 4, 8, 10, 13, 14, 18, and 19). Each of these mAbs reacted with a single CK polypeptide when tested on immunoblots of human CKs (see corresponding references in Table 1 and this paper). mAbs which could react with conformational determinants formed by two different CK polypeptides, a phenomenon described by Franke et al. (38), were not used. The most distinctive finding of the present study was expression of CK14 in the basal layers of the ectocervix, the endocervical subcolumnar reserve cells, and most cells in all squamous carcinomas examined. CK14 was not present in the endocervical columnar cells or in adenocarcinomas. Expression of CK14 detected in basal cells of ectocervix is in agreement with a designation of CK14 as a basal cell cytokeratin (22, 39, 40); however, CK14 was also detected in a few suprabasal layers of the ectocervix. The similar distribution of CK14 was detected by monospecific antiserum (22), in situ hybridization using monospecific complementary RNA probe and CK14 oligonucleotide in the epidermis (22, 41) and extension of CK14 mRNA to all cell layers was seen in the esophagus (41).

Data about the presence of a simple epithelial subset of cytokeratins (i.e., CKs 7, 8, 18, and 19) in basal cells of the ectocervix are controversial. Positive immunohistochemical reactions were obtained with some antibodies specific for CK18 [Ks 18.18 (20), (42) and Ks-B17.2 (21)] and bright staining with antibodies specific for CK19 [Km 4.62 (20) and A53-5/A2 (42)]. Bosch et al. (41) studied by immunolocalization and in situ hybridization expression of simple epithelial type cytoker-

atins in esophageal and vaginal stratified epithelium; these authors reported that positive reactions with certain antibodies to CK8, CK18, and CK19 (not defined) were occasionally seen in samples of other stratified epithelia, mostly in basal cell layers. The presence of CK19 in basal cells of oral epithelium was reported recently (43). The failure of some antibodies specific for CK18 to react with the basal cells of ectocervix, such as for example mAb RGE-53 (44), is according to the report of Franke (42), probably due to masking of specific epitopes in these cells. Interestingly, the same antibody (RGE-53) was used by Puts et al. (45, 46) who reported that SRC are CK18 negative. mAb specific for CK18 which we used in the present study (RCK 106) yielded distinct positivity in basal cells of the ectocervix (Fig. 4a). Bright staining of basal cells was obtained with antibody specific for CK19 [Km 4.62 (Fig. 4c)], similar to previously reported data (20), and rather weak staining with antibody M20 detecting CK8 [Fig. 4d]. These differences in staining intensity could be due to quantitative differences in the expression of individual cytokeratins in basal cells. We also saw weak staining of basal cells with mAb CAM 5.2, that also detects CK8 (see "Materials and Methods"); this weak positivity was no longer detectable on formalin-fixed sections, which explains why normal cervical epithelium was previously reported negative with CAM 5.2 antibody (47–49). On the basis of the absence of CAM 5.2 staining in normal ectocervix and its presence in squamous cervical carcinoma, the conclusion has been drawn (47, 48) that specific cytokeratins expressed in this carcinoma are different from those expressed in the normal ectocervix.

Cytokeratins 4, 10, and 13 we regard as suitable immunohistochemical markers of epithelial maturation in normal ectocer-
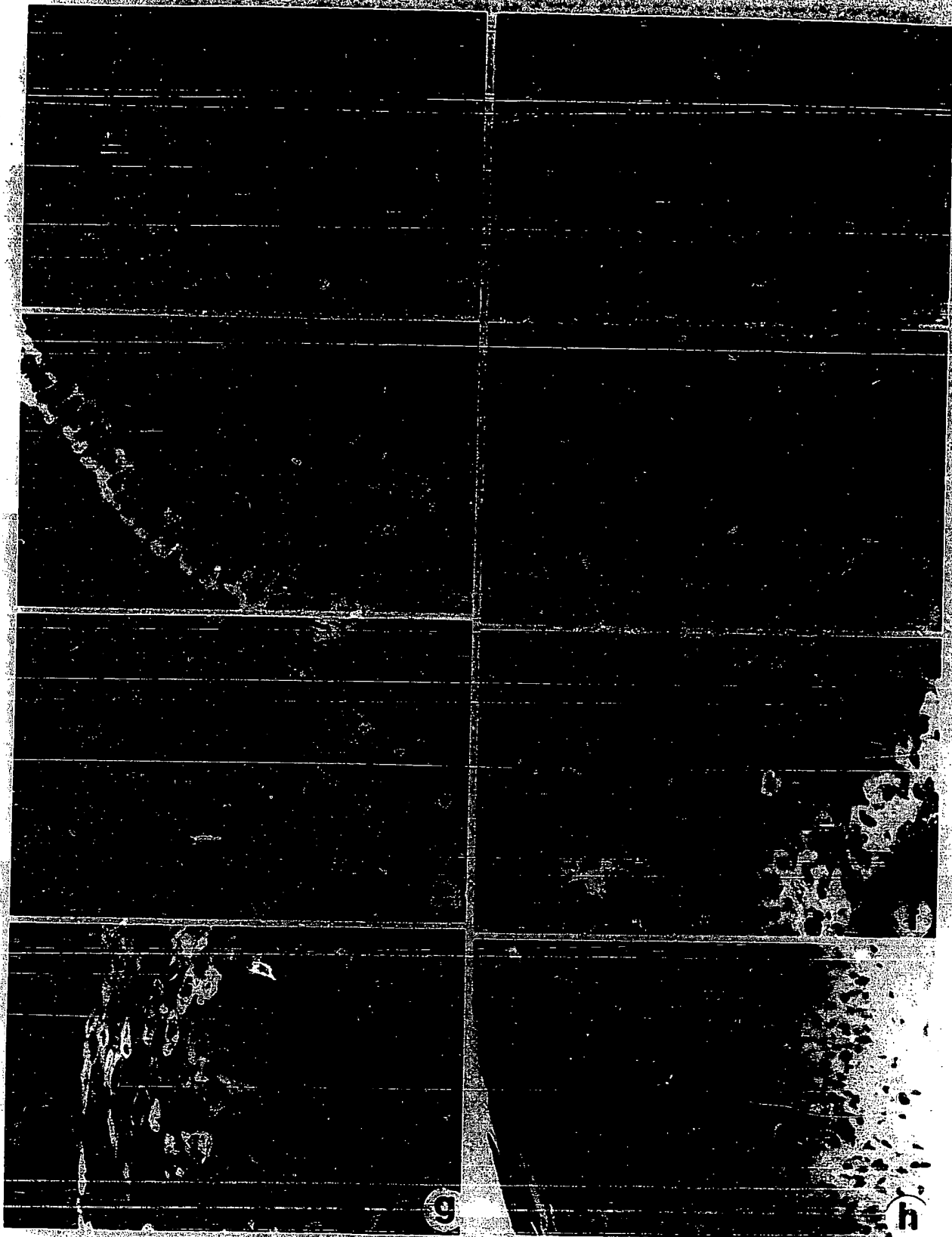
Fig. 4. Ectocervical stratified epithelium. Basal cells stained with mAbs: RCK 106, anti-CK18 (*a*); Km 4.62, anti-CK19 (*c*); M20, anti-CK8 (*d*). Basal and suprabasal cells stained with mAb DE-K14, anti-CK14 (*e, f*). Suprabasal cells stained with mAbs: 1C7, anti-CK13 (*b*); M6B10, anti-CK4 (*g*); DE-K10, anti-CK10 (*h*, immunoperoxidase technique). Note staining of CK13 in all suprabasal layers, CK4 in more superficial layers only, and a more heterogeneous pattern of staining of CK10.

Table 3 *Cytokeratin phenotype of carcinomas of the cervix uteri*

Case	FIGO[a] stage	Grade	Classification	CK4	CK8	CK10	CK13	CK14	CK18	CK19
Adenocarcinoma										
1		3	Mucin-producing	–[b]	++++	–	–	–	++++	++++
2	Ib	2	Papillary	+++	++++	–	–	–	++++	++++
3				++++	++++	–	–	–	++++	++++
SQC										
4	Ib	3	Large cell keratinizing	–	++++	–	–	++++	++++	++++
5	Ib	2	Large cell nonkeratinizing	–	++++	–	–	++++	++++	++++
6	Ib	3	Large cell keratinizing	–	++++	±	–	++++	++++	++++
7	Ib	2	Large cell nonkeratinizing	–	++w	+	±	++++	++++	++++
8	Ib	2	Large cell nonkeratinizing	–	++++	++	±	++++	++++	++++
9	Ib	2	Large cell nonkeratinizing	+	++++	++	–	++++	++++	++++
10				+	++++	+	+	++++	++++	++++
11				+++	++	+	–	+++	++++	++++
12	Ib	3	Large cell keratinizing	++++	++++	±	+++	++++	++++	++++
13	II	2	Large cell keratinizing	++	++++	+	++++	++++	++++	++++
14				++	+++	+++	++	++++	++++	++++
15	Ib	2	Large cell nonkeratinizing	±	++w	+++	++++	++++	++++	++++
16	II	3	Large cell nonkeratinizing	++++	–	+++	++++	++++	++++w	++++

[a] FIGO, Federation of International Gynecologists and Obstetricians.
[b] ++++, all or almost all tumor cells stained; +++, about 50% of cells stained; ++, 15–40% cells stained; +, small groups of stained cells; ±, single positive cells; –, negative; w, weak staining.
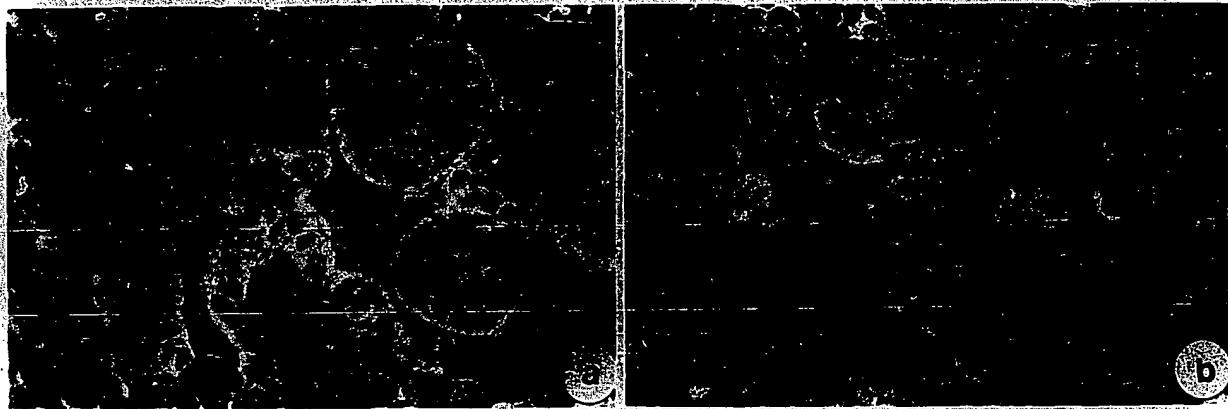

Fig. 5. Adenocarcinoma. All cells stained with mAbs: RCK 106, anti-CK18 (*a*); Km 4.62, anti-CK19 (*b*).

vix. Antibodies specific for these cytokeratins (6B10, DE-K10, and C17, respectively) all left basal cells unstained. The linkage of CK13 to squamous differentiation in several squamous stratified epithelia was reported recently (17, 42, 50). CK10 represents a major differentiation-related cytokeratin in epidermis (16, 22, 51–53). In human ectocervix its expression in a variable number of suprabasal cells was reported (30, 45, 46). *In situ* hybridization studies revealed low levels of CK4 mRNA in the basal cell layer of some stratified epithelia (18). The authors concluded that the synthesis of this protein does not depend on vertical differentiation. However, cytokeratin 4 mRNA was found to be suprabasally enriched; in immunocytochemical studies, using monoclonal antibodies specific for CK4, only suprabasal cell layers of various internal squamous epithelia were stained (17, 18, 54).

Invasive cervical squamous carcinomas revealed a complex cytokeratin pattern. Besides CKs typical for stratified epithelia, expression of simple epithelial subset of keratins (CKs 7, 8, 18) was reported in this type of tumor (47, 48, 55). In this paper we could reconfirm that "simple epithelial" cytokeratins are regularly expressed in this carcinoma. Originally, CK18 has been proposed as a useful marker to distinguish adenocarcinomas (CK18 positive) from squamous carcinomas (CK18 negative) (3, 56). This marker is not suitable for differential diagnosis of carcinomas of the human cervix uteri inasmuch as CK18 is expressed in both adenocarcinomas and squamous

carcinomas. The same holds for CK19 and CK8. Our results indicated that typing for CK14 may prove useful for differential diagnosis of cervical carcinomas, inasmuch as in our series antibody DE-K14 stained all squamous carcinomas and did not stain adenocarcinomas.

Compartmentalization of cytokeratin expression related to the state of epithelial differentiation in normal ectocervical stratified epithelium (see Table 2) was less pronounced in SQC. Basal cell-type cytokeratins (CKs 14, 18, 19, 8w) were detected in most tumor cells. Cytokeratins related to a more advanced state of epithelial differentiation in ectocervix (CKs 4, 10, 13) were detected in various numbers of cells in individual cases, from negative tumors to tumors in which most of the cells were positive for either of these cytokeratins (see case 16 in Table 3). It means that most tumor cells revealed cytokeratin phenotype of either basal cells (*i.e.*, CKs 14, 18, 19, and 8 positive; CKs 4, 10, and 13 negative) or coexpressed cytokeratins of basal and suprabasal cells; *i.e.*, the expression of basal cell cytokeratins was not down-regulated in tumor cells expressing suprabasal cytokeratins.

Typing of endocervical epithelium in this study revealed that the expression of cytokeratins in SRC is more complex than reported previously. The exact nature of SRC is not clear yet. Various possibilities of SRC origin were considered (57, 58); *e.g.*, direct derivation from columnar mucinous secretory cells, basal cells of the squamous portio epithelium, embryonal rests
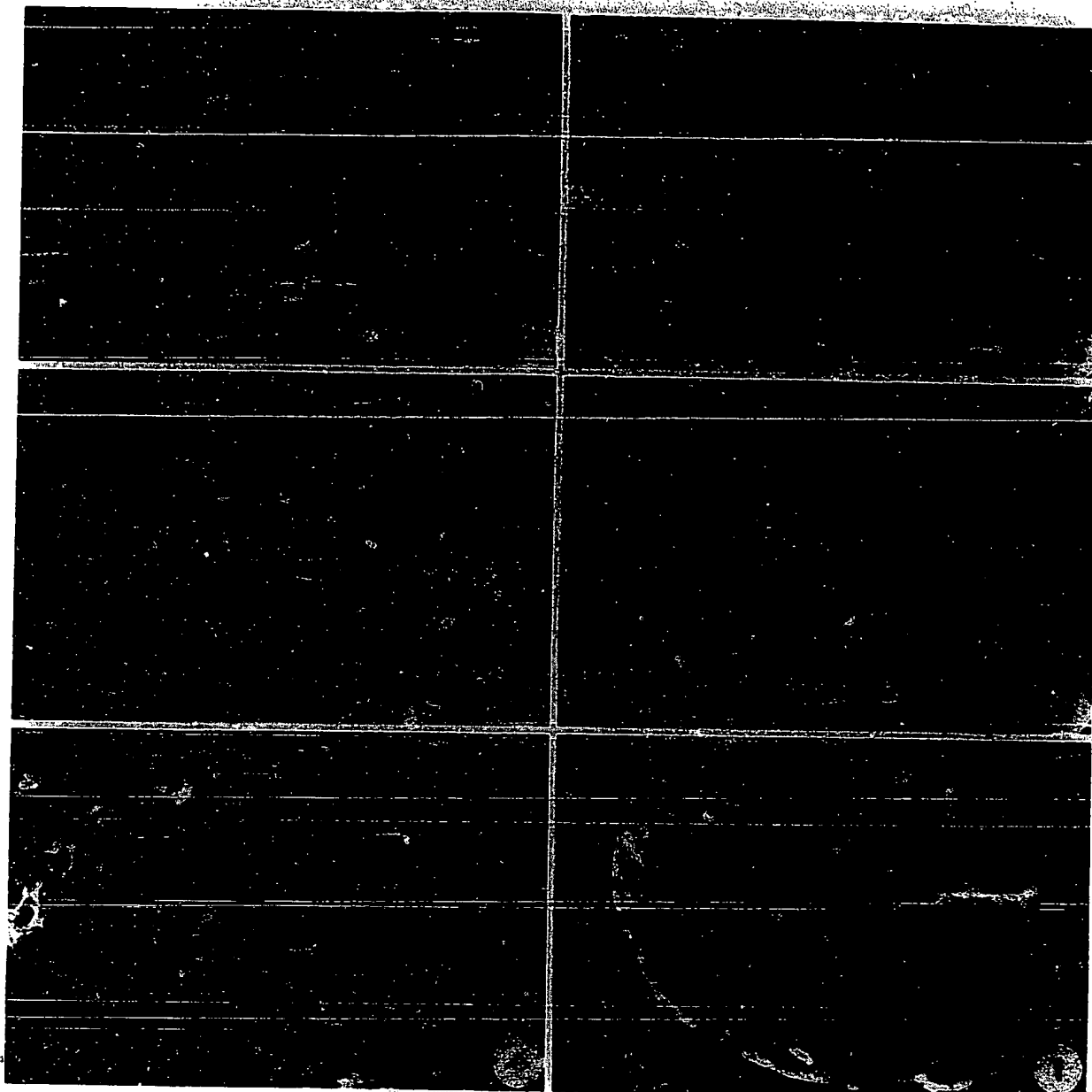
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Fig. 6. SQC. All cells stained with mAbs: RCK 106, anti-CK18 (*a*); corresponding phase-contrast micrograph (*b*); Km 4.62, anti-CK19 (*c*); M.0. anti-CK8 (*d*). Note positive staining of cells also in highly keratinized part of tumor. In some tumor islands staining of basal cell-type keratins was more pronounced on the periphery, while central parts were stained weakly or were negative: mAb DE-K14, anti-CK14 (*e*); mAb Km 4.62, anti-CK19 (*f*).

of urogenital origin, and stromal cells. The mesenchymal origin of SRC was ruled out (45) by negative reaction for vimentin and positive reaction for cytokeratin. On the basis of the finding that broad-reacting cytokeratin antibody stained both SRC and columnar endocervical cells, while an antibody specific for CK18 stained only columnar cells, a squamous nature of SRC was suggested (42, 45, 55). In the present study SRC could be stained with antibodies specific for CKs 8, 18, and 19. All these antibodies also stained columnar endocervical cells. However, we have determined a cytokeratin marker which did distinguish SRC from columnar cells. Antibody DE-K14, specific for CK14, stained brightly all SRC but did not stain columnar cells. Contrary to data published recently by another laboratory (21), we have not detected CK13 in SRC, with neither mAb 1C7 (Table 1) nor our new mAb-detecting CK13 DE-K13 (not in Table 1). It could be that the antibodies which we used recognized a specific epitope on CK13 that was masked in these

cells. An alternative explanation would be that mAb Ks-1A3 used by Levy *et al.* (21) cross-reacted with one of the basal cell cytokeratins.

Histogenesis of cervical squamous carcinoma could essentially take place by two possible pathways (Refs. 57, 59–62; see also Refs. 45, 46, and 63): (*a*) related to endocervical SRC through metaplasia; or (*b*) involving basal cells of ectocervical stratified epithelium.

In the present study all chain-specific antibodies used reacted similarly with SRC and ectocervical basal cells (see Table 2) and the cytokeratin phenotype common to SRC and basal cells of ectocervix was found in most cells of all squamous carcinomas examined. We propose the hypothesis that SRC and basal ectocervical cells are related or identical cell types, which after malignant transformation might develop squamous carcinoma. This concept unifies two above referred pathways considered for the histogenesis of cervical squamous carcinoma and cor-
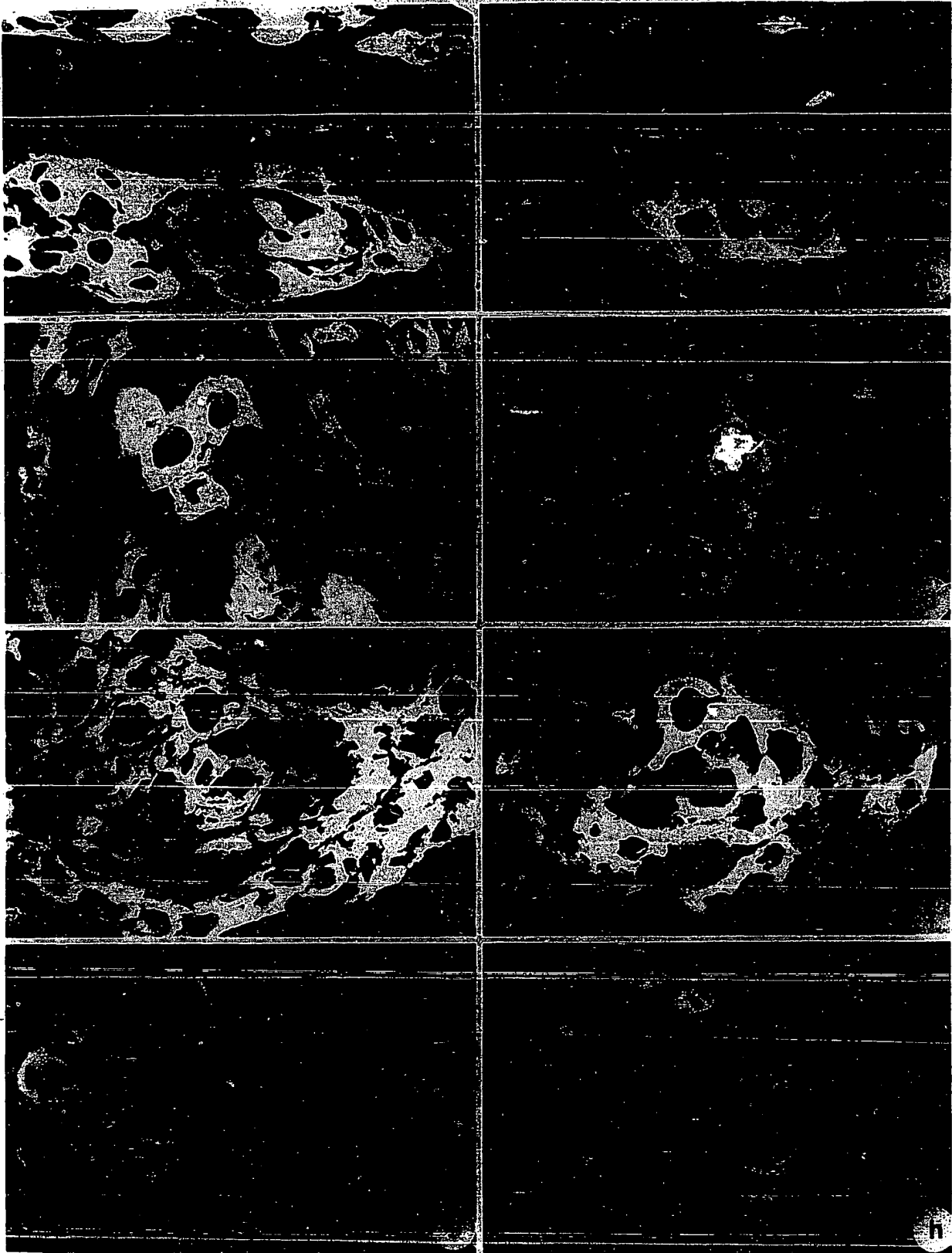
Fig. 7. SQC. More keratinized parts of tumors stained with mAbs: DE-K10, anti-CK10, (*b*, *h*); M6B10, anti-CK4 (*d*); 1C7, anti-CK13 (*f*, *g*). *b*, *d*, *f*, successive serial sections to *a*, *c*, *e*, where staining of all tumor cells is illustrated: mAb DE-K14, anti-CK14 (*a*, *c*); mAb Km 4.62, anti-CKi9 (*e*).

responds to the finding that squamous carcinoma can be involved in different anatomic locations in the human cervix.

Adenocarcinomas of the cervix uteri occur less frequent in comparison to squamous carcinoma, the reason why only a limited number of these tumors could be studied. The cytokeratin pattern of three cases analyzed previously (42, 55) by two-

dimensional gel electrophoresis was reported as CKs 7, 8, 18, and 19 positive. In our series another three adenocarcinomas were also CKs 8, 18, 19 positive and CKs 10, 13, 14 negative, similar to endocervical columnar cells that are generally considered cells of origin for cervical adenocarcinoma. The question that remains open for further study is whether certain subpopulations of columnar endocervical cells differ in their potential for malignant transformation. Single columnar cells of endocervix were CK4 positive (42) (Fig. 3e). Remarkably enough, two of three adenocarcinomas that we tested were CK4 positive. Positive staining of certain endocervical cells, indicated by electron microscopy as ciliated type, has been detected by another anti-cytokeratin mAb, Ks2.1, (21). The specificity of mAb Ks2.1 is unknown; however, the tissue distribution pattern of this antibody (in normal ectocervix stains basal layer only) is different from the distribution of CK4 (Table 2). The possibility that CK4-positive and mAb Ks2.1-positive cells represent different types of columnar cells might appear to be useful for further subdivision of cervical adenocarcinomas. The possibility that a cytokeratin 4-positive subpopulation of columnar cells has a higher potential for malignant transformation needs to be further tested.

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